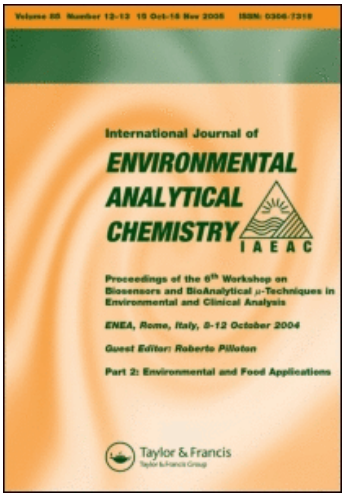

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A ratiometric fluorescent probe for zinc ions based on the quinoline fluorophore

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A ratiometric fluorescent zinc probe **1** of carboxamidoquinoline with a carboxylic acid group was designed and synthesised. Probe **1** exhibits high selectivity for sensing Zn^{2+}; about a 13-fold increase in fluorescence emission intensity and an 82 nm red-shift of fluorescence emission are observed upon binding Zn^{2+} in $EtOH/H_2O$ (1 : 1, V/V) solution. The ratiometric fluorescence response is attributed to the 1 : 1 complex formation between probe **1** and Zn^{2+} which has been utilised as the basis for the selective detection of Zn^{2+}. The analytical performance characteristics of the proposed Zn^{2+}-sensitive probe were investigated. The linear response range covers a concentration range of Zn^{2+} from 2.0×10^{-6} to 5.0×10^{-5} mol L^{-1} and the detection limit is 2.7×10^{-7} mol L^{-1}. The determination of Zn^{2+} in both tap and river water samples shows satisfactory results.

Keywords: fluorescent probe; zinc ions; quinoline; ratiometric

1. Introduction

Zinc is the second most abundant transition metal after iron in the human body. It plays an important role in various biological systems such as gene expression, protein–protein interaction and neurotransmission [1,2]. In addition, zinc is also a contributing factor in many severe neurological diseases such as Alzheimer's disease, epilepsy, cerebral ischaemia and neurodegenerative disease [2]. The total concentration of zinc can range throughout the body from nanomolar concentrations in the cytosol of certain cells to millimolar concentrations in some neuronal vesicles [3]. However, zinc is a metal pollutant of the environment: significant concentrations of zinc may reduce the soil microbial activity causing phytotoxic effect [4,5] and it is a common contaminant in agricultural and food wastes [6]. Consequently, the development of zinc selective analytical probes for biological and environmental applications is a highly active research area.

The available detection methods of Zn^{2+} are limited due to its $3d^{10}4s^0$ electron configuration not giving any spectroscopic or magnetic signals. Owing to the advantages of simplicity, high sensitivity and low cost, past decades have seen increasing interest in the

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development of fluorescent probes for zinc ions. Zinc probes operating on the principle of a simple enhancement (turn-on) or fluorescent quenching (turn-off) are widely known [7–12]. Due to the linear relationship between fluorescence intensity and analyte concentration, quantitative measurements are possible in principle. However, in most practical applications fluorescence intensity changes can also be caused by many other poorly quantified or variable factors such as the excitation intensity, dye concentration, and micro-environment around the dye (pH, polarity, temperature, and so forth) [13]. Thus, measured fluorescence intensity variation might not actually reflect differences in analyte concentration. This problem can be solved using a ratiometric probe which exhibits a large shift in its emission or excitation spectrum upon binding the analyte. The ratio of the emission intensities at two excitation or emission wavelengths is sufficient to determine the analyte concentrations, but is independent of the probe concentration, path length, or any instrument-related parameters [14]. The first ratiometric fluorescent indicator (Fura-2) was reported for selective determination of Ca^{2+} by Tsien and co-workers in 1985 [14]. Ratiometric measurement can provide precise data, thus some ratiometric probes allow quantitative detection of analyte.

Several ratiometric fluorescent probes for zinc ion have recently been described. The majority of them are based on fluorophores such as anthraquinones and coumarins [15–18], indoles and benzofurans [19,20], benzoxazoles [21], naphthalimides [22], porphyrins [13,23], benzimidazoles [24,25], cyanines [26], squaraines [27], fluoresceins [28–30], boradiazaindacenes [31], and quinolines [32–34]. However, the majority of these have low water solubility or inconvenience in preparation, only a few water-soluble and ratiometric probes for zinc have been reported. Searching for ratiometric zinc probes with good solubility is still an active field as well as a challenge for the analytical chemistry research effort.

Quinolines and their derivatives have been used traditionally as fluorogenic agents for the quantitative determinations of Zn^{2+} and other metal ions [35]. In 1987, 6-methoxy-8-*p*-toluenesulphonamido-quinoline (TSQ) was first applied for the imaging of zinc ions *in vitro* [36] and this work is regarded as a milestone in the development of fluorescent probes for biological zinc ions. In order to improve the water solubility of TSQ, carboxylic acid groups or ester groups to extend the 6-methoxyl group were introduced [37,38]. Replacing the methyl group on benzene ring with a carboxylic acid group is also an attempt to improve the solubility of TSQ [39]. However, only a few fluorescent probes for Zn^{2+} based on carboxamidoquinoline have been reported [34,40]. To improve the water solubility of zinc fluorescent probes, a ratiometric fluorescent probe for zinc ions based on carboxamidoquinoline with a carboxylic acid group (probe **1**) is reported in this paper.

Herein, we report the design, synthesis and spectral characteristics of a ratiometric zinc probe **1** with a carboxylic acid group. It shows ratiometric fluorescence response upon the addition of Zn^{2+} in 50% water/ethanol buffered at pH 7.24, which also provides a unique fluorescence response to Zn^{2+} in the presence of many other metal cations. Moreover, the feasibility of using probe **1** for the determination of Zn^{2+} in both tap and river water samples has been testified.

2. Experimental

2.1 *Reagents and chemicals*

Doubly distilled water was used throughout all experiments. Doubly distilled water was found to be zinc ion free by the method of atomic absorption spectrometry (AAS).

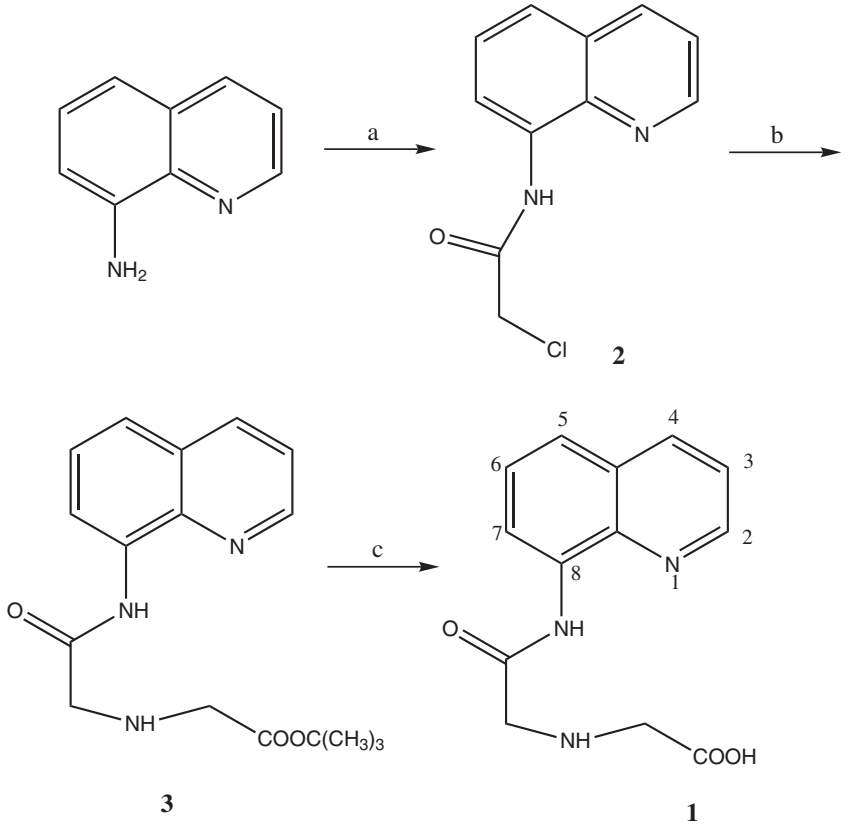
Before being used, pyridine was subjected to simple distillation from NaOH. Acetonitrile was distilled at atmospheric pressure from CaH_2. Unless otherwise stated, other chemicals were of analytical reagent grade and used without further purification or treatment.

2.2 *Syntheses*

The synthetic procedure for probe **1** is shown in Scheme 1.

Synthesis of compound 2. Compound **2** was prepared from 8-aminoquinoline and 2-chloroacetyl chloride according to a previously reported procedure [34].

Synthesis of compound 3. Compound **2** (0.2207 g, 1.0 mmol), glycine tert-butyl ester hydrochloride (1.676 g, 10 mmol), *N*,*N*-diisopropylethylamine (1.292 g, 10 mmol) and potassium iodide (0.0220 g, 0.13 mmol) were added to acetonitrile (50.0 mL). The reaction mixture were stirred and refluxed for 15 h under nitrogen atmosphere and cooled to room temperature. After the solvent was evaporated under reduced pressure, the crude product



Scheme 1. Synthesis of fluorescent probe **1**. (a) $ClCH_2COCl$, C_5H_5N, $CHCl_3$, room temperature, 2 h, 75%; (b) $H_2NCH_2COOC(CH_3)_3 \cdot HCl$, *N*,*N*-diisopropylethylamine, KI, CH_3CN, reflux, 15 h, 65%; (c) trifluoroacetic acid, CH_2Cl_2, room temperature, 24 h, 97%.

was purified by silica gel column chromatography using $CH_2Cl_2/CH_3COOCH_2CH_3$ (25 : 1, V/V) as eluent to afford a yellow solid. Yield: 0.2050 g (65%). MS (EI) *m/z*: 315.0.

Synthesis of compound 1. Compound **3** 0.1577 g (0.50 mmol) was dissolved in CH_2Cl_2. Trifluoroacetic acid (2.0 mL) was added dropwise to this solution and stirred at room temperature for 24 h. The solvent was removed by evaporation. The residue was washed three times with diethyl ether and dried in vacuo, affording compound **1** as a yellow power. Yield: 0.1258 g (97%). ^{1}H NMR (400 MHz, DMSO-d_6), δ(ppm): 10.86 (s, 1H), 8.97 (d, $J = 4.4$ Hz, 1H), 8.60 (d, $J = 7.6$ Hz, 1H), 8.45 (d, $J = 8.4$ Hz, 1H), 7.77–7.75 (m, 1H), 7.69–7.62 (m, 2H), 4.29 (s, 2H), 3.99 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ(ppm): 172.8, 169.9, 149.1, 138.1, 136.5, 134.1, 127.9, 127.0, 122.1, 121.8, 115.7, 52.5, 50.1. UV (in EtOH) $\lambda_{max} = 205$ nm($\varepsilon_{205nm} = 1.9 \times 10^4$ L mol^{-1} cm^{-1}), 238 nm($\varepsilon_{238nm} = 3.3 \times 10^4$ L mol^{-1} cm^{-1}), 311 nm ($\varepsilon_{311nm} = 5.0 \times 10^3$ L mol^{-1} cm^{-1}). MS (EI) *m/z*: 259.0. HRMS (EI) *m/z*: calculated for $C_{13}H_{13}N_3O_3$: 259.0951, found: 259.0964.

2.3 *Apparatus*

UV-vis absorption spectra were scanned on a Shimadzu MultiSpec-1501 spectrophotometer (Tokyo, Japan). All fluorescence measurements were taken on a Hitachi F-4500 Fluorescence Spectrometer (Tokyo, Japan) with excitation slit set at 5.0 nm and emission at 5.0 nm. The measurements of pH were carried out on a Mettler-Toledo Delta 320 pH meter (Shanghai, China) with a Mettler combination glass electrode (No. 4140230002). The electrode was calibrated for pH in 100% aqueous solutions using commercial pH reference solutions (pH 4.00, pH 6.86 and pH 9.18 standard solutions). The pH electrode calibration in EtOH/H_2O (1 : 1, V/V) solutions were carried out according to a previously published literature [41]. Data processing was performed on a Pentium computer with software of SigmaPlot.

2.4 *Measurement procedures*

A stock solution of 10^{-4} mol L^{-1} compound **1** was obtained by dissolving **1** in absolute ethanol.

A stock solution of 1×10^{-2} mol L^{-1} Zn^{2+} was prepared by dissolving $Zn(OAc)_2 \cdot 2H_2O$ in doubly distilled water. The stock solution of Zn^{2+} was diluted to lower concentrations of 1×10^{-3} to 1×10^{-6} mol L^{-1} stepwise.

The wide pH range solutions were prepared by adjustment of 0.05 mol L^{-1} Tris-HCl solution with HCl or NaOH solution.

The complex solution of Zn^{2+} and compound **1** was obtained by mixing 12.5 mL of the stock solution of compound **1** and 2.5 mL of Zn^{2+} solution of the different concentrations and diluting the mixture to 25 mL in a volumetric flask. In the solution thus obtained, the concentrations were 5×10^{-5} mol L^{-1} in compound **1** and 1×10^{-4} to 1×10^{-7} mol L^{-1} in Zn^{2+}. The solution was protected from light and kept at 4°C for further use. Blank solution of compound **1** was prepared under the same conditions without Zn^{2+}.

The fluorescence intensity was measured at excitation wavelength of 308 nm with the emission recorded over the wavelength range 330–600 nm. Before the fluorescence measurement, the complex solution of **1**/Zn^{2+} was allowed to stand for 10 minutes to allow complete formation of metal-ligand complex and the response time for zinc concentration $\leq 6 \times 10^{-6}$ mol L^{-1} is less than 5 min.

3. Results and discussion

3.1 *Spectral characteristics*

Fluorescence and UV-vis absorption spectra were used to study the model of coordination of **1** with Zn^{2+} before the application of **1** for the detection of Zn^{2+}. The fluorescence emission spectra were recorded from 5×10^{-5} mol L^{-1} solution of probe **1** at 25°C in CH_3CH_2OH–H_2O (1 : 1, V/V) and Tris-HCl (0.05 mol L^{-1}) buffer (pH 7.24). Figure 1 shows the effect of Zn^{2+} concentration on the fluorescence emission spectra of probe **1**. As can be seen from Figure 1, the fluorescence emission spectra of probe **1** are sensitive to Zn^{2+}. With the stepwise addition of Zn^{2+} to a solution of probe **1**, the fluorescence emission of probe **1** at 402 nm decreases while that at 484 nm increases. An isoemission point appears at near 411 nm. In the presence of excess Zn^{2+}, about a 13-fold increase in fluorescence emission intensity and an 82 nm red-shift of fluorescence emission from 402 to 484 nm were observed. Its emission intensity ratio at 484 and 402 nm enhanced with the increasing zinc concentration. These results provided a proof for the formation of a coordination complex of compound **1** with Zn^{2+}, which constituted the basis for the determination of zinc concentration with probe **1** by the fluorescence ratiometric method. For a control experiment, the fluorescence emission spectra were measured from 5×10^{-5} mol L^{-1} solution of probe **1** in the absence and presence of 5.0×10^{-5} mol L^{-1} Zn^{2+} in 100% water buffered at pH 7.24 (Figure 2). From Figure 2, one can see that probe **1** exhibited better sensitivity in 50% water/ethanol than in 100% water. Taking into consideration the sensitivity, a 50% water/ethanol buffered at pH 7.24 was chosen in this paper.

In order to further understand the binding mode of **1** and Zn^{2+}, the UV-vis spectra of **1** in the absence and presence of Zn^{2+} were investigated (Figure 3). The absorption spectrum of probe **1** exhibits an absorption peak at 239 nm and a broad band around 306 nm. Absorption experiments show that upon zinc binding there was a 43 nm red-shift from 306 to 349 nm of absorption wavelength. These spectral characteristics illustrated the

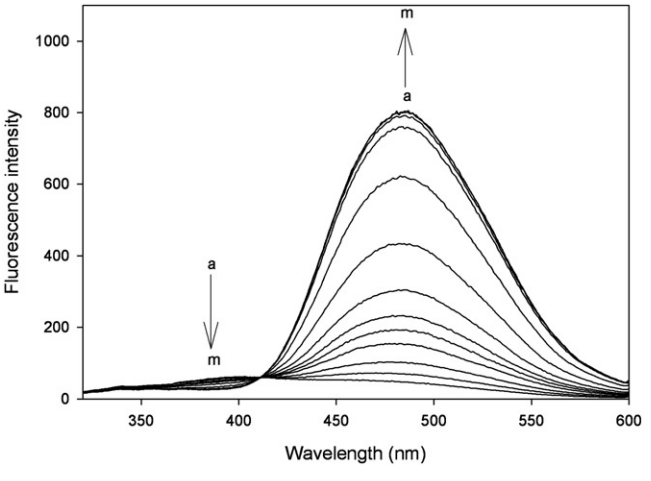


Figure 1. Emission spectra of probe **1** (50 μM) in the prescence of various concentrations of Zn^{2+}: 0, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 80 μM from a to m. These spectra were measured in 0.05 M tris-HCl buffer (ethanol/water = 1 : 1, V/V, pH 7.24). The excitation wavelength was 308 nm.

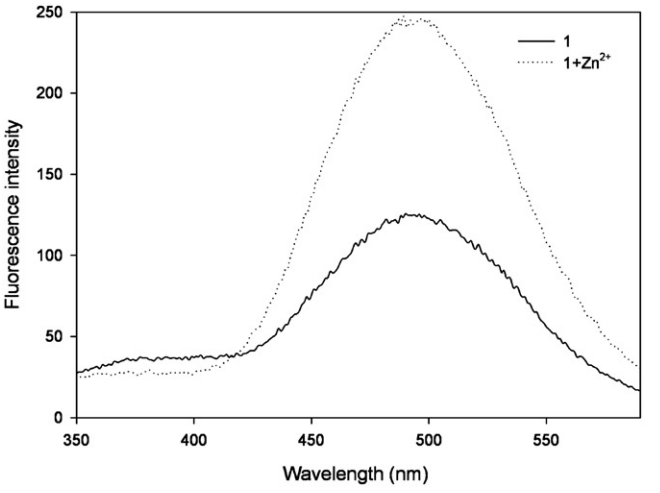


Figure 2. Emission spectra of probe **1** (50 μM) before (—) and after (···) the addition of $Zn(OAc)_2 \cdot 2H_2O$ (50 μM). These spectra were measured in 0.05 M tris-HCl buffer (100% water, pH 7.24).

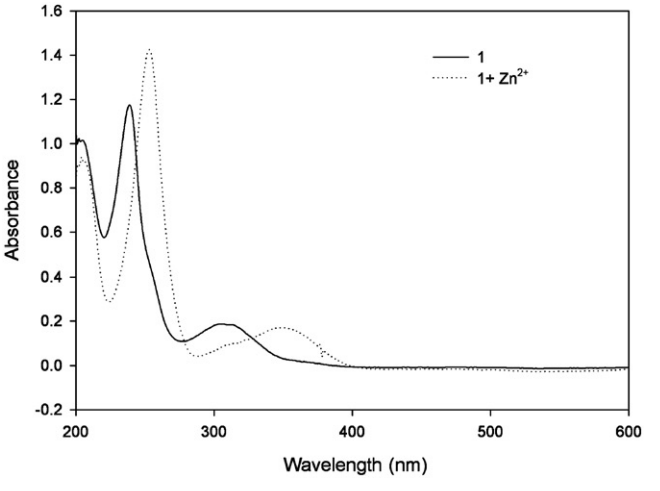
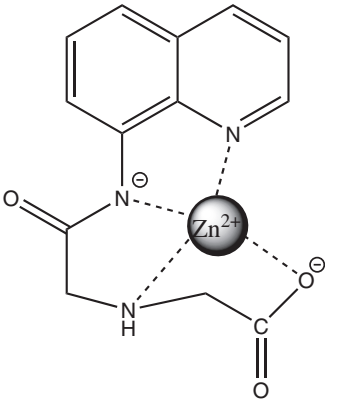


Figure 3. Absorption spectra of probe **1** (50 μM) before (—) and after (···) the addition of $Zn(OAc)_2 \cdot 2H_2O$ (50 μM). These spectra were measured in 0.05 M tris-HCl buffer (ethanol/water = 1 : 1, V/V, pH 7.24).

transformation from free **1** to the **1**/Zn^{2+} complexes. ^{1}H NMR studies further revealed the coordination patterns between probe **1** and Zn^{2+}. It is noted that the chemical shift of 2-position H atom of quinoline shifted downfield to $\Delta\delta = 0.10$ ppm upon titration of excess of zinc suggesting the coordination of quinoline N to zinc ion. Meanwhile, the chemical shifts of other aromatic protons showed evident chemical shift changes upon completely binding zinc indicating the involvement of N atom of 8-imino of probe **1** in Zn^{2+} coordination. The proton signal of $-CH_2-$ in $COCH_2NH$ group was shifted to lower field by 0.01 ppm suggesting that N atom in $COCH_2NH$ group might coordinate with



Scheme 2. Possible coordination mode for probe **1** with Zn^{2+}.

Zn^{2+}. The ^1H NMR data of probe **1** in D_2O are as follows: ^1H NMR (400 MHz, D_2O), δ(ppm): 8.84 (d, $J = 4.4$ Hz, 1H), 8.38 (d, $J = 8.4$ Hz, 1H), 8.15 (d, $J = 6.8$ Hz, 1H), 7.83 (d, $J = 8.0$ Hz, 1H), 7.65–7.57 (m, 2H), 4.26 (s, 2H), 3.74(s, 2H). When probe **1** bound with zinc completely, the ^1H NMR data in D_2O are as follows: ^1H NMR (400 MHz, D_2O), δ(ppm): 8.94 (d, $J = 4.8$ Hz, 1H), 8.77 (d, $J = 8.4$ Hz, 1H), 8.08 (d, $J = 3.6$ Hz, 1H), 8.02 (d, $J = 8.4$ Hz, 1H), 7.84–7.81 (m, 1H), 7.79–7.75 (m, 1H), 4.27 (s, 2H), 3.74 (s, 2H). The FT-IR spectra of compound **1** and **1**-Zn(II) complex were also studied to determine the binding mode of probe **1** and Zn^{2+}. When probe **1** completely coordinated with Zn^{2+}, the peaks at 3244, 2798, 2646, 2474 cm^{-1} which correspond to the dimeric aborptions of O–H in carboxyl group disappeared, and the peaks at 1552 and 1454 cm^{-1} which are consistent with the characteristic absorption of carboxylate moiety appeared. The IR data support that a binding participation of the carboxyl group occurs with zinc ions. Thus, a possible coordination mode for probe **1** with Zn^{2+} was proposed (Scheme 2).

In this paper, the fluorescence quantum yield was determined using rhodamine 6G in EtOH ($\Phi_F = 0.94$) as standard at an excitation wavelength of 500 nm, and the fluorescence quantum yield is calculated using the following Equation (1) [42].

$$\phi_{unk} = \phi_{std} \frac{(I_{unk}/A_{unk})}{(I_{std}/A_{std})} \left(\frac{\eta_{unk}}{\eta_{std}}\right)^2 \quad (1)$$

where ϕ_{unk} and ϕ_{std} are the radiative quantum yields of the sample and standard, I_{unk} and I_{std} are the respective integrated emission intensities of the corrected spectra for the sample and standard, A_{unk} and A_{std} are the respective absorbances of the sample and standard at the excitation wavelength (500 nm in all cases), and η_{unk} and η_{std} are the indices of refraction of the corresponding solvents of the sample and standard solutions. Thus, the fluorescence quantum yield of probe **1** in EtOH/H_2O (1 : 1, V/V) solution is 0.009. Probe **1** shows larger enhancements (14.4-fold in Φ_F) upon completely coordinating Zn^{2+}. The mechanism by which this chelation-enhanced fluorescence occurs at 484 nm is that intramolecular hydrogen bonds of 8-aminoquinoline were broken upon zinc coordination, and some of the non-radiative transitions between electronic states were suppressed [34,40,43], thus enhancing fluorescence emission. The large red shifts in both emission and absorption wavelength of probe **1** after binding Zn^{2+} ions can be explained in terms of the

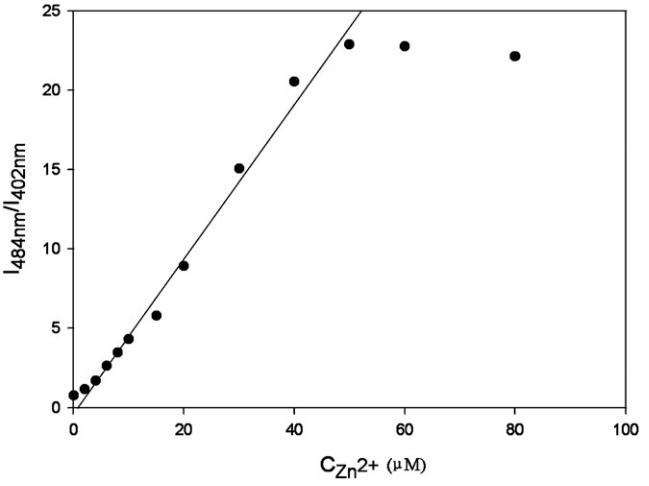


Figure 4. Fluorescence ratio I_{484nm}/I_{402nm} of probe **1** (50 μM) as a function of Zn^{2+} concentration in 0.05 M tris-HCl buffer (ethanol/water = 1 : 1, V/V, pH 7.24). The excitation wavelength was 308 nm.

possible intramolecular charge transfer (ICT) mechanism [34,40,44]. When a fluorophore contains an electron-donating group conjugated to an electron-withdrawing group, it undergoes ICT from the donor to the acceptor upon excitation by light. If the electron-withdrawing group within the fluorophore interacts with a cation, the latter enhances the electron-withdrawing character of the group, stabilising the excited state and therefore causing a red shift in the fluorescence maximum. In the case of probe **1**, coordination between zinc ions and the nitrogen atom of the quinoline ring further strengthens the ICT process therefore causing the observed large red shift in the maximum of the fluorescence spectrum.

3.2 *Principle of operation*

Figure 4 shows the dependence of emission intensity ratios between 484 and 402 nm (I_{484nm}/I_{402nm}) on the concentration of Zn^{2+}. As shown in Figure 4, I_{484nm}/I_{402nm} increased linearly with the concentration of Zn^{2+} (0–1 equiv) up to a mole ratio (**1**/Zn^{2+}) of 1 : 1, and there it remained unchanged. From the fluorescent titrations, the binding constant of Zn^{2+} in EtOH/H_2O (1 : 1, V/V) solution was determined to be $4.5 \times 10^6\,M^{-1}$ [45]. The linear response of I_{484nm}/I_{402nm} toward C_{Zn}^{2+} was obtained in zinc concentration range of 2.0×10^{-6} to $5.0 \times 10^{-5}\,mol\,L^{-1}$. The ratiometric calibration line can be expressed by the following Equation (2):

$$I_{484nm}/I_{402nm} = -0.3542 + 0.4863\ C_{Zn}^{2+}\ (R = 0.9950). \quad (2)$$

Here I_{484nm} and I_{402nm} are the fluorescence emission intensity of probe **1** actually measured at a given metal concentration at 484 and 402 nm, respectively. C_{Zn}^{2+} represents the concentration of zinc ion added. The detection limit obtained for Zn^{2+}, estimated by $3\,s_B/m$ (where s_B is the standard deviation of 10 measurements of the blank and m is the slope of the calibration line) was $2.7 \times 10^{-7}\,mol\,L^{-1}$ [46,47].

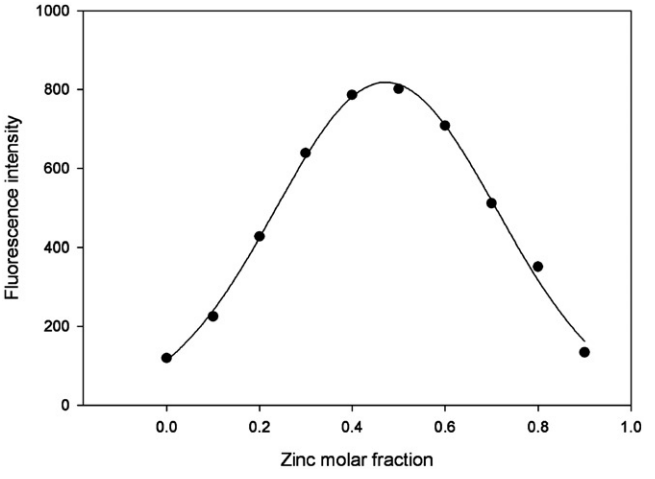


Figure 5. Job's plot for probe **1** in 0.05 M tris-HCl buffer (ethanol/water = 1 : 1, V/V, pH 7.24). The total concentration of **1** and Zn^{2+} was 100 μM. The excitation wavelength was 308 nm.

Figure 4 also implied the formation of a **1**/Zn^{2+} adduct of 1 : 1 stoichiometry between the probe **1** and Zn^{2+}. Moreover, a Job's plot using a total concentration of 100 μM probe **1** and Zn^{2+} showed a maximum emission intensity at 484 nm when the molecular fraction of Zn^{2+} was close to 0.5, which also confirmed the 1 : 1 complexation of probe **1** and Zn^{2+} according to a previously reported literature [48] (Figure 5).

3.3 *Effect of pH*

The effects of pH on the fluorescence intensity of probe **1** in the absence or presence of 5.0×10^{-5} mol L^{-1} Zn^{2+} were investigated at a pH range from 2.05 to 12.51 (Figure 6). As shown in Figure 6, probe **1** exhibits good fluorescence response to Zn^{2+} in the range of pH from 2.05 to 12.51. Probe **1** has no appreciable fluorescence sensing ability to Zn^{2+} at pH values lower than 2.05, which might be caused by the protonation of the imino group of probe **1** at lower pH values resulting in a weak coordination ability of Zn^{2+} [34]. However, probe **1** shows satisfactory Zn^{2+}-sensing ability in the range of pH from 2.05 to 12.51. At pH = 7.24, the emission intensity ratio between free **1** and **1**/Zn^{2+} reached its maximum value of 13.0. Taking into consideration the sensitivity and response speed, a 0.05 M Tris/HCl buffer solution at pH 7.24 was chosen as optimum experimental condition.

3.4 *Selectivity*

The fluorescence responses of probe **1** to various cations and its selectivity for Zn^{2+} are illustrated in Figure 7. The concentration of Zn^{2+} was fixed at 5.0×10^{-5} mol L^{-1}. Cations were added as chlorides, nitrates, acetate and sulfates. As can be seen from the black bars in Figure 7, I_{484nm}/I_{402nm} significantly enhanced upon the addition of Zn^{2+} and slightly decreased upon binding to Cu^{2+}, Co^{2+} and Ni^{2+}. Moreover, the addition of other cations did not affect I_{484nm}/I_{402nm} of the fluorescent probe **1**. Cu^{2+}, Co^{2+} and Ni^{2+} can not shift the spectrum of probe **1** in the same way as Zn^{2+}, but they quenched the emission intensity

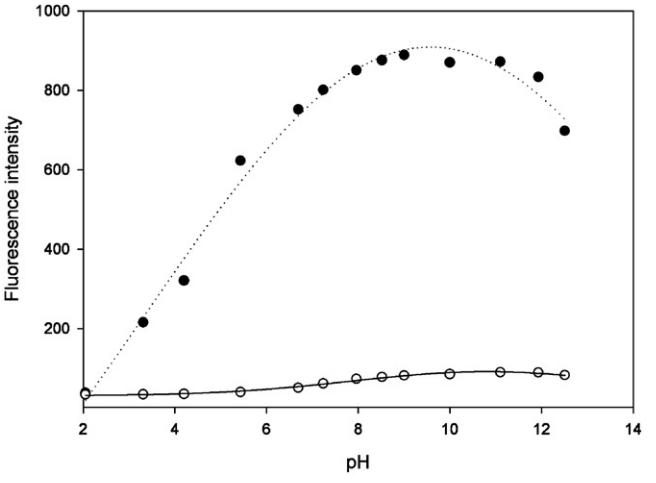


Figure 6. Effect of pH on the fluorescence intensity of 50 μM probe **1** in the absence (clear circles) or presence (filled circles) of 50 μM Zn^{2+}. All data were obtained at various pH values (pH 2.05–12.51) and the excitation wavelength was 308 nm.

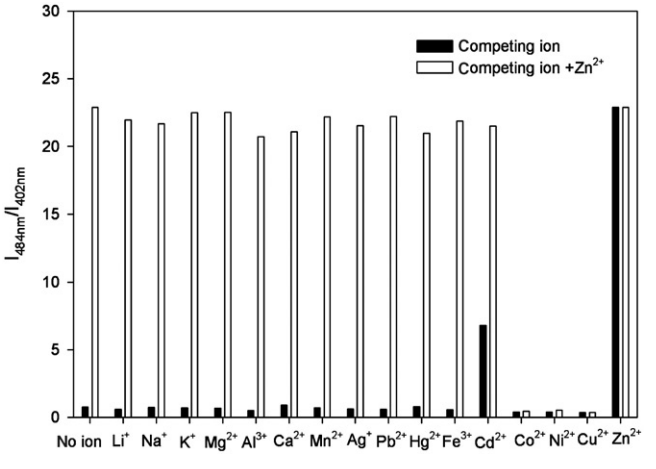


Figure 7. Metal ion selectivity of probe **1** (50 μM). All data were obtained at pH 7.24 (0.05 M Tris/HCl) and were expressed as the fluorescence ratio (I_{484nm}/I_{402nm}). The excitation wavelength was 308 nm. Cations (10 equiv. relative to probe **1**) were added as $Li_2SO_4 \cdot H_2O$, $Co(CH_3COO)_2 \cdot 4H_2O$, $NiCl_2 \cdot 6H_2O$, $Mn(CH_3COO)_2 \cdot 4H_2O$, NaCl, $Al_2(SO_4)_3 \cdot 18H_2O$, $Pb(NO_3)_2$, KCl, $Ca(CH_3COO)_2 \cdot H_2O$, $CdCl_2 \cdot 2.5H_2O$, $HgNO_3 \cdot H_2O$, $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, and $MgSO_4$. Other cations were added as $Zn(CH_3COO)_2 \cdot 2H_2O$ (50 μM), $Pb(NO_3)_2$ (100 μM), and $AgNO_3$ (100 μM). Measured at 25°C in CH_3CH_2OH–H_2O (1 : 1, V/V) and Tris-HCl (0.05 mol L^{-1}) buffer (pH = 7.10). Black bars: each cation was added. White bars: each cation and zinc ion were added.

at both 484 nm and 402 nm resulting in slight decrease of I_{484nm}/I_{402nm}. Cd^{2+} shifted the spectrum of probe **1** in the same way as Zn^{2+}, but the magnitude of I_{484nm}/I_{402nm} is less than that for Zn^{2+}. In order to further test the interference for other common cations on the determination of Zn^{2+}, a competition experiment was performed in which the fluorescent probe was added to a solution of Zn^{2+} in the presence of other metal ions

Table 1. Results of Zn^{2+} determination in tap and river water samples with probe **1**.

	Sample	Concentration[a] (M)	Concentration[b] (M)	Relative error (%)
Tap water	1	$(3.02 \pm 0.12) \times 10^{-6}$	$(3.14 \pm 0.09) \times 10^{-6}$	3.97
	2	$(3.10 \pm 0.10) \times 10^{-6}$	$(3.18 \pm 0.13) \times 10^{-6}$	2.58
River water	1	$(1.24 \pm 0.05) \times 10^{-5}$	$(1.22 \pm 0.03) \times 10^{-5}$	−1.61
	2	$(1.28 \pm 0.04) \times 10^{-5}$	$(1.32 \pm 0.02) \times 10^{-5}$	3.12

Notes: [a]Determined by atomic absorption spectrometry.
[b]Average of three determinations found by the proposed method.

(white bars in Figure 7). The competition experiments show no significant variation in the emission intensity ratio (I_{484nm}/I_{402nm}) except Cu^{2+}, Co^{2+} and Ni^{2+}. Thus, probe **1** exhibits nice selectivity for Zn^{2+} over other common cations except Cu^{2+}, Co^{2+} and Ni^{2+}. Fortunately, the three cations would have little influence in living systems, since they exist at very low concentrations [26]. But with a detection limit of 10^{-7} M, the present probe **1** might not work for the determination of zinc in biological systems where the free zinc has a concentration in 10^{-9} M regime.

3.5 *Preliminary analytical application*

In order to examine the applicability of the proposed method in practical sample analysis, the probe **1** was applied in the determination of zinc ion in both tap and river water samples and the results were compared with those given by the atomic absorption spectrometry reference method. The river water samples were obtained from Xiang River. Results are shown in Table 1. The concentration of Zn(II) was determined using the calibration curve given in Figure 4. From Table 1, one can see that the content of zinc ions in tap and river samples as determined by probe **1** was in good agreement with that obtained by atomic absorption spectrometry with a relative error less than 5%. Thus, the present probe seems useful for the determination of Zn^{2+} in real samples.

4. Conclusion

A ratiometric fluorescent probe **1** for Zn^{2+} based on quinoline has been developed. It shows remarkable sensitivity and selectivity for the determination of zinc in $EtOH/H_2O$ (1 : 1, V/V) solution. The fluorescence emission of probe **1** at 402 nm decreases while that at 484 nm increases upon the addition of zinc and emission intensity ratios between 484 and 402 nm (I_{484nm}/I_{402nm}) are selective for Zn^{2+}. A coordination complex of 1 : 1 stoichiometry between probe **1** and Zn^{2+} was formed. The probe **1** can be applied to the quantification of Zn^{2+} with a linear range covering from 2.0×10^{-6} to 5.0×10^{-5} mol L^{-1} and the detection limit is 2.7×10^{-7} mol L^{-1}. The probe **1** has been used for the determination of Zn^{2+} in both tap and river water samples. The method proposed in this work is simple, rapid, sensitive and selective.

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